Evaluation of Phytochemical Composition and Antibacterial Properties of Crude Extracts from Anchote (*Coccinia abyssinica* (Lam.) Cogn)

M.Sc. THESIS

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Evaluation of Phytochemical Composition and Antibacterial Properties of Crude Extracts from Anchote (*Coccinia abyssinica* (Lam.) Cogn)

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As thesis research advisors, we hereby certify that we have read and evaluated the thesis prepared by Tadele Tekilu under our guidance, which is entitled "Evaluation of Phytochemical Composition and Antibacterial Properties of Extracts from Anchote (Coccinia abyssinica (Lam.) Cogn**)**" We recommend that it could be submitted as it fulfills the requirements for the M.Sc. thesis.

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Final approval and acceptance of the thesis is contingent upon submitting the final copy to the Council of Graduate Studies (CGS) through the Graduate Committee (DGC) of the school of biological sciences and biotechnology.

DEDICATION

This thesis manuscript is dedicated to my beloved mother Desta Dilgassa for her unbound love; patience and strength that helped me come to this level.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is a result of my genuine work and that all sources of materials used for this thesis have been duly acknowledged. This thesis has been submitted in partial fulfillment of the requirements for M.Sc. degree at Haramaya University and will be deposited at University Library to be available to borrowers under the rules of the library. I declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author was born on September 2, 1984 G.C in East Wallaga Zone, Wayu Tuka Woreda, Oromia Regional State, Ethiopia. He attended his Elementary, Junior Secondary School and preparatory school at Konchi Catholic School and Nekemte Preparatory school, respectively. After completion of his Preparatory Education at Nekemte Secondary School in 2001, he joined Ambo University Department of Biology and graduated on July 9, 2003 E.C. with B.Sc. degree in Biology. Then, he was employed as a senior technical assistant at Haramaya University. He served the Haramaya University to this capacity until he joined the Postgraduate Program Directorate of Haramaya University to pursue his M.Sc. degree in Microbiology.

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ACRONYMS/ ABBREVIATIONS

ANOVA	Analysis of Variance
CRD	Completely Randomized Design
EBDI	Ethiopian Biodiversity Institute
EPHI	Ethiopian Public Health Institution
MIC	Minimum Inhibitory Concentration
NLSI	National Laboratory Standard Institute
NA	Nutrient Agar
SPSS	Statistical Package for Social Sciences
TAE	Tannic Acid Equivalent
WHO	World Health Organization
MHA	Mueller Hinton Agar
TPC	Total Phenolic Content
ATCC	American Type Culture Collection
GAS	Group A *streptococcus*
STEC	Shiga toxin-producing" *E. col*

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ABSTRACT

The over increasing antibiotic resistance of pathogens has driven a global initiative towards the search for new sources of antimicrobial substances mainly from medicinal plants. Based on the existing traditional claim, this study investigated the antimicrobial activities and phytochemical composition of crude extracts of anchote (Coccinia abysinica) leaf and tuber using extracting solvents such as ethanol and hexane against the gram positive (Streptococcus aureus, Staphylococcus pyogene) and gram negative (Salmonella typhi, Escherichia coli) pathogenic bacteria. The antibacterial activity was tested against four bacterial strains by agar well diffusion method. The minimum inhibitory concentrations (MIC) of the plant extracts against the test pathogens were assessed using the broth dilution method. The antibiotic amoxicillin was used as positive and distilled water as negative controls for bacterial pathogens. The ethanol tuber and hexane leaf extracts did not show inhibition zone, while the tuber and leaf extracts (ethanol and hexane) showed the highest activity against the tested bacterial strain. The hexane tuber extracts and ethanol leaf extracts of Coccinia abyssinica showed inhibitory effects at a concentration of 200mg/ml with zones of inhibition ranging from10.50 -14.50mm and 12.17-15.83mm, respectively. The lowest and highest zones of inhibitions were observed against Salmonella typhi and Staphylococcus aureus respectively. The inhibitory activities of the crude extracts were significantly different at ($p<0.05$). Phytochemical analysis showed that the extracts contained tannin and terpenoids in both tuber, leaf (ethanol and hexane) extracts. Alkaloid, tannin and terpenoids in tuber (ethanol, hexane) extract, while tannin, terpenoid, saponin, flavonoid and steroids only in leaf (ethanol, hexane) extracts. This study showed the evaluation of Phytochemical Composition and Antibacterial Properties of Extracts from Anchote (Coccinia abyssinica (Lam.) Cogn).

Keywords: *Anchote accession, Coccinia abyssinica, antimicrobial activities*

1. INTRODUCTION

Medicinal plants (MP) have been used as sources of medicine in almost all human cultures starting from the time when the early man became aware of his environment. Today, traditional medicines of plant origin have been expanded globally and used widely in both developing and developed countries. Different records show that people have used herbal medicinal plants to treat different diseases, and medicinal plants represent a rich source of antimicrobial agents. For example, about 80% of Ethiopian people depend on traditional medicine of plant origin due to cultural acceptability, affordability and accessibility of the remedy (Kebede *et al.*, 2006).

Plants that are used medicinally in different countries are known for their potent chemicals with medicinal properties (Desta *et al.*, 2005). Therefore, medicinal plants are believed to be important sources of new chemical substances with potential therapeutic effects (Davis, 2004). As evidence to this, an impressive number of modern drugs have been isolated or derived from natural source mainly from plants based on their use in traditional medicine (Girma and Dereje, 2015). Thus, plants are valuable sources of nutrients and bioactive compounds for treatment of common infectious diseases (Aliyu *et al.*, 2008; Khan *et al*., 2010; Hossain *et al*., 2014). Bioactive compounds in fruits, vegetables, herbs, spices, pulses, cereals, and starchy plant foods also provide the best protection against the development of chronic illness such as cancer, cardiovascular diseases, type II diabetes, hypertension, cataract and impaired cognitive function (Halvorsen *et al.*, 2002; Del Rio *et al*., 2013). Therefore, because of their therapeutic effect, plants are used to synthesize many useful drugs in which about 80% of the medicines are directly or indirectly obtained from them (Yadav *et al*., 2010).

Anchote (*Coccinia abyssinica*) is the only tuberous cucurbit belonging to the family Cucurbitaceae (Girma & Hailu, 2007). It is endemic to the South and South Western parts of Ethiopia (Amare, 1973), mainly in the Western region of Ethiopian highlands such as in Eastern Wollega, Western Wollega, Kelam Wollega, and Mattu (Westphal, 1974). The most widely used vernacular name is Anchote, spelt “Ancootee” in Affan Oromo. It is also called: “Ushushu” (in Welayita), “Shushe” (in Dawuro), and “Ajjo” (in Kafigna) (Demel *et al.*, 2010). It exists both in wild and cultivated, and its cultivation is mainly for food where the tubers are boiled and consumed like many other root and tuber crops (Fufa and Urga, 1997). Traditionally, boiled after

peeling or boiled before peeling and/or further cooking are applied prior to consumption. Beasides its food value, it is also regarded as medicinal plant due to the fact that it is used traditionally to cure people having bone-fracture or joint dislocation (Abera, 1995; Endashaw, 2007; Girma and Dereje, 2015; Habtamu and Kelbessa, 2016). According to Abera (1995), Anchote became popular in the custom of Oromo and non-Oromo peoples because of its medicinal role gained from practical experience than the rest of its uses. The high medicinal value of Anchote tuber is possibly because of its high calcium and protein content (Amare, 1973). The over matured Anchote tuber "*guboo*", though no scientific research has proved it, is presumed to contain high nutrient concentration especially calcium and iron in its tuberous root, *and* thus used for healing many health maladies (Girma and Dereje, 2015). Dawit and Estifanos (1991) reported that the juice prepared from anchote tuber can be used to treat gonorrhea, tuberculosis and cancer suggesting that the juice contains some active phytochemicals with antimicrobial properties (Habtamu and Kelbessa, 1997). Today there are some challenges related to infectious diseases.

There has been an alarming increase in the incidence of new and re-emerging infectious diseases (Bhatia *et al*., 2012). Human pathogenic bacterial are becoming resistance for commonly available antibiotics (Omulo *et al*., 2015). Due to this researcher need to discover new compounds with diverse chemical structures and good sources for drugs. *Anchote (Coccinia abyssinica)* is one of medicinal plants mainly in the Western and south western region of Ethiopia. Different parts of a*nchote (Coccinia abyssinica)* uses for the treatment of the chronic illness like: Cancer, cardiovascular diseases, hypertension, diabetes, wounds, Ulcers and inflammation (Halvorsen *et al*., 2002). As well as leaves of anchote treatment of skin disease, wound and inflammation. These plants are the most naturally effective and cheapest sources of drugs (Prince and Prabakaran, 2011).

However, no enough study has evaluated the antimicrobial property and secondary compounds content of the tuber and leaves of this plant. With these points in mid, we hypothesized that anchote leaves and tubers contain secondary compounds and help in fighting some human bacterial pathogens. This study was, therefore, designed to answerer whether or not anchote tuber and leaves contain secondary compounds of antimicrobial properties with the following general and specific objectives.

General objective

- To look the activities of the major secondary compounds and evaluate the antimicrobial activities of crude extracts from tuber and leaves of anchote (*Coccinia abyssinica*).

Specific objectives

- To detect the selected secondary compounds from crude extracts of anchote tubers and leaves;
- To evaluate antimicrobial activities of tuber and leaves extracts of anchote against *Salmonella typhi* (ATCC 13311), *E. coli* O157 H:7 (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogene* (ATCC 19615)
- To determine the minimum inhibitory concentration (MIC) of crude extracts of tuber and leaves of anchote.

2. LITERATURE REVIEW

2.1. Historical Background of Medicinal Plants

Traditional medicin*e* (TM) is defined as the sum total of the knowledge and practices weather explicable or inexplicable, used in the diagnosis, prevention and elimination of physical, mental and social imbalances (Davis, 2004). It has incorporated plant, animal and mineral based medicines, spiritual therapies, including techniques and exercises applied in sole or in combination (Davis, 2004). It depends exclusively on past practical experience and observations handed down from generation to generation, verbally or in writing. And also comprises therapeutic practices that have been in existence often for hundreds of years before the development of modern scientific medicine and are still in use without documented evidence of adverse effect (Elujoba *et al.,* 2005).

Medicinal plants have been used as sources of medicine in virtually all cultures. During the last decade, the use of traditional medicine (TM) has expanded globally and is gaining popularity. It has continued to be used not only for primary health care of the poor in developing countries, but also in countries where conventional medicine is predominant in the national health care system (Gupta *et al.,* 2008). According to WHO, 2014 herbal medicines serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries.

In Ethiopia, traditional remedies represent not only part of the struggle of the people to fulfill their essential drug needs but also they are integral components of the cultural beliefs and attitudes (Ahmed *et al.,* 2005). The Ethiopian flora is estimated to contain between 6500 and 7000 species of higher plants of which about 12% are endemic. Ethiopia is also a home for many languages, cultures and beliefs that have in turn contributed to the high diversity of traditional knowledge and practice of the people, which, among others include the use of medicinal plants. More than 95% of traditional preparations in the country are of plant origin (Ahamed and Mohamed, 2005). Some of the common uses of the medicinal plants sold in markets include fumigation, vermifuge, pain relief and treating skin infections. Antimicrobial and wound healing plants are among some of the major medicinal plants that are commonly available in markets

(Dagne, 2007). Medicinal plants have played a significant role in ancient traditional systems of medication in many countries. Indigenous herbs are used as remedies against various diseases in the traditional system of medicine or in ethno medical practices (Kupta, 2008). There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases and drug resistance pathogenic bacteria.

2.2. Traditional Medicine in Ethiopia

Traditional medical practitioners mostly implement herbs, spiritual healing, bone-setting and minor surgical procedures in treating disease. Ethiopian traditional medicine is vastly complex and diverse and varies greatly among different ethnic groups. Most traditional medical practices in Ethiopia rely on an explanation of disease that draws on both the “mystical” and “natural” causes of an illness and employ a holistic approach to treatment (Bishaw, 2013). Under the rule of Menelik (1865-1913) Western medicine became significantly more incorporated into the Ethiopian medical system. Numerous medical envoys from abroad, starting with the Italians and Russians, were influential in building hospitals, providing medical training and participating in vaccination campaigns. However, most medical establishments primarily served the urban elites and foreign missionaries and were concentrated in the major cities (Yadav *et al*., 2010).

Despite Western medicine becoming more widespread in Ethiopia, Ethiopians tend to rely more on traditional medicine. Conventional medical services remain concentrated in urban areas and have failed to keep pace with the growing population, keeping health care access out of reach for most Ethiopians living in Ethiopia. Because traditional medicine is culturally entrenched, accessible, and affordable, up to 80% of the Ethiopian population relies on traditional remedies as a primary source of health care (Bishaw *et al.*, 2013). Moreover, Western medicine has become more focused on preventative measures and people seeking curative practices still rely on indigenous medicine as the primary source for health care.

2.3. Common medicinal plant used in Ethiopia for treatment of disease

About 80% of individuals from developing countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties safety and efficiency. In the local traditional setting, plant parts such as roots and leaves are used without recourse to phytochemical isolates (Bishnu *et al.,* 2005). *Coccinia abyssinica* root and leaf is effective in treating anti-inflammatory, used to heal chronic wounds, diabetes and cancer. It is believed to remove toxins from the body neutralizing free radicals and purify the blood (Habtamu *et al*., 2015).

Traditionally herbal medicinal plants are mostly used to treat infections caused by *Streptococcus agalactiae*. Desta *et al., (2005*) show the inhibitory activities of avocado extracts against some bacterial pathogens. *Streptococcus agalactiae* is also inhibited by Croton *floribundus, Cupania vernalis* and other Brazilian plants (Juneja *et al*., 2007). Extracts from leaf, flower and root part of *Catharanthus roseus* show high inhibition zone against these bacteria (Juneja *et al*., 2007). Leaves, stems and flowers extracts of *Acacia aroma* had also antimicrobial activities against *staphylococcus aureus* (Aliyu *et al*., 2014).

According to a study conducted by Turker *et al*. (2009) about 22 plant species are screened for antibacterial activity against *Aeromonas hydrophila, Yersinia ruckeri, Lactococcus garvieae, Streptococcus agalactiae* and *Enterococcus faecalis*. The result indicates that among the tested plants 11 species are capable to inhibit the growth of *S. agalactiae* with high zone of inhibition. A study done in Bishoftu, Ethiopia, describe antimicrobial properties of steem, bark of *Combretum molle* and leaves of *Xanthium strumarium* against *Staphylococcus aureus* and *Streptococcus agalactiae* (Habtamu *et al*., 2015). *Cenchrus ciliaris, Brachiaria sp, Abutilon indicum, Coccinia grandis, Asteracantha longifolia, Trichodesma indicum, Dactyloctenium indicum, Spermacoce hispida* have an inhibitory effect on *Staphylococcus aureus* (Doss *et al*., 2012).

2.4. Uses of Anchote (*Coccinia abyssinica*)

Anchote [*Coccinea abyssinica* (Lam.) Cogn.] is an endemic root and tuber crop of Ethiopia cultivated for human consumption (Abera, 1995; Beruk & Fikre, 2015; Habtamu, 2014; Tilahun *et al*., 2014). It is grown widely in the Western and South-Western parts of the country. It belongs to cucurbitaceae family, one of the major families from the plant kingdom encompassing about 115 genera and 960 species (Sawadogo *et al.*, 2012). Anchote is a vine like cucurbit with a high yield and short crop cycle used as an important dietary & medicinal plant (Girma and Hailu, 2007; Yassin *et al., 2013*). The crop is known for its tuberous root and tender leaves which are used for food (Abera, 1995).

Anchote has a significant contribution for the cultural and social values of the Oromo people like during the meskel holy day, during the weeding and on different cermony (the largest community in Ethiopia) since long ago (Abera, 1995; Desta, 2011; Daba *et al*. 2012). The plant has been grown over a wide range of environments for a long time, and its cultivation and utilization have been passed from generation to generation through oral tradition, with very little recorded information (Girma and Hailu, 2007). The unique characteristics of the plant are the edibility of its different parts such as its tuber, leaf and fruit which makes the plant ideal as potential food security crop (Amare, 1973; Endashaw, 2007; Desta, 2011). For instance, the protein content of Anchote tuber ranges from 4.6-16.4% (Desta, 2011) which is high compared to other root and tuber crops commonly consumed in Ethiopia, whose protein values range from 1-2% (Gebremedhin *et al*., 2008).

Studies indicate that Anchote has relatively higher crude protein, utilizable carbohydrate, crude fiber, energy, and ash content compared to sweet potato, potato and cassava (Habtamu *et al*. 2013). The calcium content of Anchote is also reportedly high (Desta, 2011; Habtamu *et al.*, 2013). Traditionally, Anchote is consumed to heal broken or fractured bones as well as to strengthen sick people, which could be attributed to its high, high calcium content (Abera, 1995; Habtamu & Kelbessa, 1997). *Coccinia abyssinica* is a phytotherapic plant used in folk medicine. Leaves of this plant are used for treatment of number of ailments including diabetes, wounds, ulcers and inflammation.

2.5. Importance of secondary metabolites for Human Health

Secondary metabolites are the non-nutritive bioactive compounds produced by plants through various metabolic pathways. The various secondary metabolites commonly present in plants are steroids, terpenes, phenolic compounds and alkaloid (Czinner *et al.*, 2001). These compounds are reported to have anti-mutagenic, anti-carcinogenic, antioxidant, antimicrobial and anti-inflammatory potentials (Desta & Deans, 2000; Pizzale *et al.*, 2002; Sokovic *et al. 2002;* Lampe 2003; Srinivasan, 2005). Plants commonly use these compounds for self-defense and to enhance their survival period (Williams, Stone, Hauck, & Rahman, 1989). Therefore, plants are rich sources of these phytochemicals that can protect against oxidative stress and thus attracting attention recently due to their potential antioxidant properties and their marked effects in chemoprevention of various diseases (Abubakar *et al.*, 2014).

2.5.1. Flavonoids

Flavonoids are the most common phenolics found in plants tissues often responsible for blue, purple, yellow, orange and red color formation together with the carotenoids and chlorophylls (Khoddami, Wilkes, & Roberts, 2013). The flavonoid family includes flavones, flavanols, iso flavones, anthocyanins, anthocyanidins and proanthocyanidin (Gujjeti & Mamidala, 2013; Rong, 2010). The chemical nature of flavonoids depends on their structural class; degree of hydroxylation, other substitutions and conjugations, and degree of polymerization are known to be synthesized by plants to microbial infection (Heim, 2002). The other beneficial effects of flavonoids are improved blood flow, the inhibition of cholesterol absorption and protection from damage by ultraviolet radiation (Taura *et al*., 2014)

2.5.2. Saponins

Saponins are a group of secondary metabolites of steroid or tri terpene glycoside compounds in a variety of plants, which have many benefits. Many saponins have detergent properties, form astable foam in aqueous solutions, show hemolytic activity and have bitter taste. It forms colloidal solutions in water, and foamy like soap if mixture is shaken. Saponins as a group include compounds that are glycosylated steroids, tri terpenoids and steroid alkaloids (Kumar *et al., 2011).* The presence of saponins is characterized by the existence of a stable colloidal solution (Astuti, 2011). Many saponins are known to be antimicrobial, to inhibit mould and to

protect plants from insect attack. Saponins may be considered a part of plants' defense systems, and such have been included in a large group of protective molecules found in plants named phyto anticipins or Phyto protectants (Cheynier *et al*., 2005).

2.5.3. Alkaloids

Alkaloids are diverse group of low molecular weight phytochemicals found in 20% of the plant species. The different subclasses of alkaloids include: betalain, indole, isoquinoline, lycopodium, pyrrolidine, pyrrolizidine and quinolone (Penula & Zahidah, 2001). The significant pharmacological activities of alkaloids reported are analgesic (morpheine and codiene), anticancer (taxol), anthiarrythmic (ajmaline), gout suppressant (colchicine), muscle relaxant (tubocurarine), and antibiotic (sanguinarine) (Fufa, 2007).

2.5.4. Terpenes

Terpenes are the largest classes of phytochemicals that include mono terpenoids, iridoids, sesqui terpenoids, sesquiterpene lactones, dieterpenoids, triterpenoids, phytosterols, saponins, saponins glycosides and carotenoids. These phytochemicals exist in green leafy vegetables, fruits and grains. Plants use triterpenes for carbon fixation through photosynthesis and protection from diseases involving chronic damage and growth dyes regulation. Animals use these phytochemicals for hormonal and growth regulatory functions. Terpenes possess antioxidant, antimicrobial and antiviral properties (Obadoni *et al*., 2002).

2.6. Some selected human bacterial pathogenic species

Bacteria are all around us, in the air, on objects and normally found in and on human body. When bacteria are on the human body in the absence of disease, it is called a colonizer. However, people can get infected from pathogenic bacteria from food, water, abrasions and other wounds and even from colonizing bacteria if it gets into a normally sterile part of the body. Pathogenic bacteria are the ones that cause disease to the host. The list of pathogenic bacteria is quite large, but there are some that are common causes of human diseases (Dorman and Deans, 2000).

2.6.1. *Staphylococcus aureus*

Staphylococcus aureus is one of the most common causes of human disease. Most staphylococci colonize the skin and mucous membranes of people without disease. However, given the right conditions, staphylococci can cause superficial and systemic infections. Some of the more common superficial infections caused by *S. aureus* include boils, impetigo and folliculitis. More serious and common infections caused by this organism are pneumonia, bacteremia, infections of the bone and wounds. *Staphylococcus aureus* can also produce toxins that can cause such diverse diseases as food poisoning and toxic shock syndrome. Other species of staphylococci that commonly cause disease in humans is *Staphylococcus saprophyticus*. This is a common cause of urinary tract infections in sexually active women (Dorman and Deans, 2000).

2.6.2. *Streptococcus pyogenes*

Streptococcus pyogenes are also called group A *streptococcus* (GAS). They are gram-positive extracellular, a non-sporing coccoid-shaped bacterium that grow in chains with large colonies greater than 0.5 mm in size. GASs are small in size which is less than 2 µm in length. They are facultative anaerobe, non-motile, negative for catalase, salt tolerant and oxidase and susceptible to bacteriocines. Erythrogenic, toxin Streptolysin O, Streptolysin S, Hyaluronidase, DNase, NADase, Streptokinase and M Protein endotoxin are used as virulence factors for *S. pyogenes* (Elujoba and Siragusa, 2015)

Streptococcus pyogenes are identified as a causative agent for skin diseases such as Impetigo, cellulitis and erysipelas (Cogen *et al., 2008*). Impetigo is a contagious infection which forming pustules and yellow crusty sores. It usually occurs on the face, neck and hands of young children and infants. Impetigo is more rarely in adults. There is a different type of impetigo named as non-bullous impetigo (Impetigo contagiosa), Bullous impetigo and Ecthyma. Non bullous usually begin with red sores around the nose and mouth. These blisters burst, leaving a weeping, red rash that becomes crusted. This rash may be itchy but is not painful. Bullous impetigo is most common in children under age two. Blisters usually appear first on the torso, arms, and legs. These blisters may initially appear clear and then turn cloudy and can last longer than blisters caused by another type of impetigo.

The areas around the blisters may be red and itchy. Ecthyma is the most serious form of impetigo because it affects the second layer of the skin, rather than just the top layer. Blisters tend to be painful and may turn into ulcers or aggravated, open sores. The symptoms are red sores that pop easily and leave yellow crust fluid-filled blisters itchy rash skin lesions swollen lymph nodes (Silverberg and Block, 2008). Many plants are used as TM to treat infection caused by *S. pyogenes*. Leaves, stems and flowers of *Acacia aroma, Azadirachta indica,* the 3, 4-dihydroxycinnamic acid isolated from leaves of *Cassia alata* (Paul *et al*., 2013), ethanol extracts of ginger (*Zingiber officinale*) (Sebiomo *et al.,* 2010), leaf and root of *Lantana indica,* methanol crude extracts of *Scaevola spinescens,* have antibacterial activity against *S. pyogenes* (Mejin, 2009). Leaf and gel of *Aloe vera* also have antimicrobial activity against *S. pyogenes (*Prince and Prabakaran, 2011).

2.6.3. *Escherichia coli O157:H7*

Escherichia coli are a bacterium that normally lives in the intestines of humans and animals. Although most types of these bacteria are harmless, several produce toxins that cause illness. Some strains of *E. coli,* including *E. coli* O157:H7, produce toxins known as Shiga toxins and are called "Shiga toxin-producing" *E. coli* (STEC). These may cause severe diarrhea and kidney damage. *E. coli* O157:H7 was first identified as a possible human pathogen in 1975 in a California patient with bloody diarrhea and was first associated with a food borne (ground beef) outbreak of disease in 1982. This serotype (defined by its O and H surface antigens) and some non-O157 serotypes of *E. coli* produce toxins, also called Shiga-like toxins because of their similarity to toxins produced by *Shigella dysenteriae* (Ellin, 2006).

E. coli O157:H7 is a toxin producing bacteria that causes intestinal disease in people which lasts about one week. Diarrhea with blood is typical. Severe cases can lead to kidney problems which *E. coli* O157:H7 causes a wide spectrum of human diseases, including bloody and non-bloody diarrhea, hemorrhagic colitis (HC), occasional kidney failure and hemolytic uremic syndrome (HUS) and *E. coli* O157 contamination of drinking, surface and recreational water has emerged as an important cause of human disease (Chalmers *et al*., 2000; Elder *et al*., 2001). There is a varying level of antibiotic resistance among *E. coli* O157:H7 isolates. Some *E. coli* O157:H7

isolates have resistance to one or more antibiotics whereas others are multidrug resistant (Einas *et al*., 2015).

2.6.4. *Salmonella typhi*

The Salmonellae belong to a genus of the family Enterobacteriaceae. They are gram negative, facultative anaerobic and non-spore forming rods. Motile forms have peritrichous flagella. They are usually catalase positive, oxidase negative and reduce nitrates to nitrites (Adams and Moss, 2008). Salmonellae are recognized as a major cause of enteric fever and gastroenteritis. Many foods, particularly those of animal origin, have been recognized as vehicles for transmitting the organisms to human and to the food processing and preparation environment (Behailu, 2006). *Salmonella* typhi is a strain of bacteria that lives only in humans. It causes a bacterial infection of the intestinal tract and occasionally the bloodstream which is called typhoid fever. *Salmonella typhi* bacteria are shed in the urine or stool of infected persons, including chronic carriers. *Salmonella typhi* is spread by eating or drinking contaminated food or water or by contact with stool from infected persons.

2.7. Minimum Inhibitory Concentration

The minimum inhibitory concentration is defined as the lowest concentration able to inhibit any visible bacterial growth on the culture plates. This was determined from readings on the culture plates after incubation. The most commonly employed methods are the tube dilution method and agar dilution methods. Serial dilutions were made for bacterial growth media. The test organisms are then added to the dilutions of the products, incubated, and scored for growth. This procedure is a standard assay for antimicrobials. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. MIC is generally regarded as the most basic laboratory measurement of the activity of an antimicrobial agent against an organism. Clinically, the minimum inhibitory concentrations are used not only to determine the amount of antibiotic that the patient will receive but also the type of antibiotic used, which in turn lowers the opportunity for microbial resistance to specific antimicrobial agents (Zahidah and Noriham, 2013).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

This study was conducted in the laboratories of the Haramaya University, School of Biological Sciences and Biotechnology. Phytochemical analysis and extraction were carried out in General Laboratory while antibacterial activity tests were carried out in Microbiology Laboratory, Haramaya University (HU) main campus. HU is located at about 510 km East of Addis Ababa, and geographically, it is located at latitude of 09°.0N and longitude of 42°.0 E with an altitude of 1950 meters above sea level. It has a moderate average tempreture of 16^{o}C and the mean maximum and minimum annual temperature is 24.02 and 9.73^{o}C respectively. The mean annual rain fall is 780 mm (Seifemichael, 2014).

Plant samples were collected from East Wollega, Wayyu Teka, Wera babo Migna kebele. East Wollega is one of the Zones in Oromia region. The town and cities in this zone include Nekemte. Nekemte,West Ethiopia which is located at 300 Km from Addis Ababa. It has latitude and longitude of 9°5′N 36°33′E and an elevation of 2,088 meters above sea level**,** annual rain are 760-1016mm and the maximum temperature of 39.0 ^{o}C (Tilahun, 2014).

3.2. Plant Sample collection and Extract Preparation

Fresh leaf and different anchote tubers were selected fron local or different cultivars of Anchote (*Coccinia abyssinica*) were collected from East Wollega, Oromiya Regional State. Ten different anchote tubers were selected fron local or different cultivars depend on the maturity or anchote tubers which kept more than two years in the soil. Healthy tubers from each accession were harvested, washed, peeled and sliced to small pieces and mixed thoroughly in order to prepare 400g of samples. These were placed in a paper bag and dried to a constant weight in a hot air oven (DHG- 9055A, Momment, Germany) set at 105^{o}C. Similarly, 200g bunches of newly growing tips of leaves were first cleaned and chopped in to small pieces. Leaves were air dried under shade. The oven dried Anchote tubers and leaf samples dried under shade were then milled using an electrical miller (FW 100, Yusung Industrial Ltd, and China) to fine powder to pass a mesh size of 0.425mm. Finally, the dried and powdered samples were packed in paper

bags and sealed in an airtight polyethylene bag, and labeled before storing in a refrigerator set at 4^{o}C for further analysis.

The dried powders were extracted separately by maceration using 97% ethanol and hexane as solvents. For this, 100gm of powder and 300ml of ethanol/hexane were added to 500ml Erlenmeyer flasks and the mixtures were mixed very well by shaking. Then, the flasks were wrapped up by aluminum foil to avoid contamination. The mixtures were then shaken on a platform shaker for 3 days at room temperature. After filtration using Whatman no. 1 filter paper, aliquots of the extracts were concentrated by heating on a hot plate at about 30 - 40 ºC for 30 min for some of the qualitative analysis, whereas the rest were left at room temperature to dry for seven days. The dried (or smi-dried) extracts were then preserved at 4ºC until used for some qualitative and quantitative analyses, and for anti-bacterial activity test (Biswas *et al*., 2013; Taura *et al*., 2014; Shah and Yadav, 2015).

3.3. Analysis of major Secondary compounds

3.3.1. Qualitative determination of major secondary compounds

The following standard procedures, preliminary identification of phytochemical constituents including alkaloids, flavonoids, saponins, steroids, tannins and terpenoids was done using either the powder or the extracts of the test plant.

Test for tannins (Ferric chloride test): To a 2 ml of extract, few drops of 1% Lead acetate was added and formation of a yellowish precipitate was taken as indication for the presence of tannins (Savithramma *et al*., 2011).

Test for phlobatannins: 2g of each solid extract were placed into separate test tubes and mixed with 20 ml of distilled water. The mixture was boiled in a water bath for 10 min. After cooling, each mixture was filtered separately through a Whatman No 1 filter paper. Thereafter, 2 ml of 1% aqueous hydrochloric acid was added to each mixture and shaken to develop red precipitate that indicates the presence of phlobatannins (Ajayi *et al*., 2011).

Saponins (Foam test): To 2 ml of the filtered both tubers by (ethanol and hexane) and leaf (ethanol and hexane) extract, 5ml of distilled water was added to a test tube. The mixture was shaken vigorously to observe for the appearance of stable persistent froth on warming, as preliminary evidence for the presence of saponins (Abba *et al*., 2009).

- **Foam test:** 2.5 ml of filtrate was added to test tube and diluted to 10 ml with distilled water and shaken vigorously for 2 minutes. Formation of froth confirmed the presence of saponin in the filtrate.
- **Emulsion test:** 2 drops of olive oil were added to the frothing and the mixture shaken vigorously for a few minutes. Formation of a fairly stable emulsion indicated the presence of saponin.

Test for flavonoids (Alkaline reagent test): To a filtrate of 2ml of concentrated both tubers by (ethanol and hexane) and leaf (ethanol and hexane) extract, dilute ammonia (5 ml) and concentrated H_2SO_4 (1 ml) were added slowly and the development of a yellow color that disappears on standing was considered as an indicator for the presence of flavonoids (Ayoola *et al*., 2008).

Test for steroids (Lieberman-Burchard's Test): 2 ml of chloroform and 10 drops of acetic acid were placed in test tube. The concentrated tuber (ethanol and hexane) and leaf (ethanol and hexane) extract (0.5 ml) was added to the test tube and mixed with the solvents. Then, 2 ml of concentrated sulphuric acid was added along the side of test the tube. The change of red color through blue to green serves as an indicator for the presence of steroids (Edeoga and Kiruba, 2014).

Test for terpenoids (Salkowski test): Five ml of each extract was mixed with 2 ml of chloroform, and concentrated H_2SO_4 (3ml) was carefully added to form a layer. Formation of a reddish brown coloration at the interface was considered as a positive indicator for the presence of terpenoids (Edeoga *et al.,* 2005).

Test for alkaloids (Mayer's test): 2ml of 1% HCl was added to 4.5 ml of each concentrated ethanol and hexane extracts in different test tubes. Each mixture was heated for 2 min in a water bath while stirring continuously. It was then cooled and filtered. The resulting filtrate was tested with Mayer's Reagent for the presence of alkaloids as described by (Obadoni *et al.,* 2002). That

is, 1ml of the filtrate was added to 0.4 ml of Mayer's reagent and formation of cream yellow precipitate was monitored as an indication for the presence of alkaloids.

3.3.2. Quantitative analysis of major secondary metabolites

The powders and preserved solidified extracts of *Coccinia abyssinica* were used for standard quantitative estimation of the major secondary metabolites as indicated below.

Total phenol determination: Spectrophotometric method was used to quantify total phenol content in anchote leaves and tubers extract as described by Cogen *et al*. (2012). For this, stock solutions of extracts (1 mg/ml, w/v) were prepared by dissolving 10 mg of the solidified extracts in 10 ml of 80% ethanol. Then, 500µl of the stock extract was transferred to test tube and mixed with 500µl of the Folin-Ciocalteu solution and 1 ml of the sodium carbonate solution in a test tube. The final volume was then adjusted to 10 ml by adding 8 ml of distilled water. The sample solutions were kept at room temperature for 30 minutes and their absorptions were measured at 560 nm using distilled water as a blank.

The total phenolic content was calculated as tannic acid equivalent (TAE) by the following equation described (Mohamed *et al*. 2011).

TPC = C*V/M

Where: C- is the concentration of tannic acid established from the calibration curve in mg/ml, V- is the volume of the extract solution in ml and M- is the weight of the extract using in gram.

For quantification of the absorbance, tannic acid standard was used to calibrate standard curve. For this, Stock solution of tannic acid (0.1 mg/ml, w/v) was prepared by dissolving 10 mg of tannic acid in 100 ml of 80% ethanol. Then, 0.1, 0.2, 0.3, 0.4 and 0.5 ml of stock solutions were pipetted and transferred into separate pint flasks. 500µl of 10% Folin-Ciocalteu solution was added to each of the pint flasks and mixed homogeneously for 10 seconds. Then, they were allowed to stand for 5 minutes. Thereafter, 1ml of 7.5% Sodium carbonate was mixed homogeneously for 30 seconds. Then, the final volume was adjusted to 10 ml with distilled water in order to obtain the final standard tannic acid concentration of 1, 2, 3, 4 and 5µg/ml. These standard reaction mixtures were allowed to stand for 30 minutes after which their absorptions

were measured at 560nm using distilled water as a blank. Calibration curve was constructed from obtained data.

Total alkaloid determination: Harborne (1973) method was used for quantification of total alkaloid. For this, the powder (3g) was added into a 50 ml Erlenmeyer flask. Then, 20 ml of 10% acetic acid in ethanol was added into the flask and the solution was allowed to stand for 4 hrs being covered. Next, the solution was filtered and concentrated ammonium hydroxide was added drop wise to the filtrate until the formation of precipitate stopped or completed. The whole solution was allowed to settle the precipitate. Then, the precipitate was collected, and washed with dilute ammonium hydroxide and then filtered. The obtained residue was dried and weighed after complete dryness and alkaloid content was calculated as mg per gram of the sample powder used.

Saponin determination: Crude saponins determination was done according to Obadoni & Ochuko (2002) and Edeoga *et al*. (2005). Briefly, 20g of sample powder was mixed with 100ml 20% aqueous ethanol was added. The mixture was then heated over a hot water bath for four hr with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 10 ml 20% ethanol. After concentrating the combined extracts to 40 ml at about 90°C over water bath, the concentrated extract was then transferred into a 250-ml separatory funnel and 20 ml of diethyl ether was added followed by vigorous shaking until the aqueous and ether layers are separated. By discarding the ether layer, the aqueous layer was collected repeatedly until no more layer formation observed. To the collected aqueous extract, 60 ml n-butanol was added and washed twice with 10 ml of 5% aqueous sodium chloride. Finally, the remaining solution was evaporated in a water bath and dried in an oven until constant weight was obtained and the saponin content was calculated.

Total terpenoid determination: About 2 g of powder was soaked in 50 ml of 97% ethanol for 24 hrs. The extracts were filtered by Whatman No 1 filter paper and the filtrate was added in to separating funnel followed by addition of 50 ml of petroleum ether. The resulting mixture was shaken and allowed to stay for 5 minutes for layer formation. Then, the bottom layer was drained and discharged while top petroleum ether was collected and concentrated to dryness using rotary evaporator at 40 ºC stay for 18 hrs. The mass of dried extract was considered as crude terpenoids, and expressed as mg per the sample powder used (Furguson, 1956).

3.4. Antibacterial Susceptibility Test

3.4.1. Media Preparation and Standardization of Inoculum

3.4.2. Inoculation of Nutrient Agar (NA) plates

First nutrient agar media was prepared by dissolving the required amount of nutrient agar (NA) powder with distilled water. Then the mixture was heated on hot plate and autoclaved to get boiled and uniform solution. The resulting autoclaved medium was transferred into separate Petri plates and allowed to solidify. Each of the selected bacterial pathogens such as *Salmonella typhi* (ATCC 13311), *E. coli* O157 H:7 (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogene* (ATCC 19615) obtained from Ethiopian Public Heath Institution (EPHI) and Ethiopian Biodiversity Institution (EBI) were cultured on separate nutrient agar plates and incubated for 24 hr at 37°C to obtain colonies.

For the preparation of bacterial inoculums, two to three bacterial colonies cultured on NA plate were picked up with a sterile inoculating loop and transferred into a test tube containing sterile normal saline solution and vortexed thoroughly. This was repeated until the turbidity of each bacterial suspension matched the turbidity of 0.5 McFarland Standards as described by the Clinical Laboratory Standards Institute. The resulting suspension was then used as inoculums of the test pathogen in the antimicrobial susceptibility test.

3.4.3. Preparation of different concentrations of the crude extracts

The stock solutions (200 mg/ml) of the crude extracts were prepared by reconstituting 2g of each of the dried extracts in 10ml of distilled water. Different concentrations (100 mg/ml, 150mg/ml and 200mg/ml) of each of the extracts were then prepared from their respective stocks. For preparing the 100mg/ml,150mg/ml, and 200mg/ml, concentrations, 5.5ml, 6.5ml and 7.5 ml of the different stock solutions of the extracts were transferred to separate 10ml capacity volumetric flasks and the flasks were filled up to 10 ml mark with distilled water (Alabi *et al*., 2012).

3.4.3. Inoculation of Mueller Hinton Agar (MHA) plates with test pathogens and antimicrobial assay.

First Mueller Hinton Agar (MHA) was prepared by dissolving the required amount of MHA powder with distilled water. Then the mixture was heated on hot plate and autoclaved to get boiled and uniform solution. The resulting autoclaved medium was transferred into separate Petri plates and allowed to solidify. Within 15 minutes of adjusting the turbidity of the suspension of inoculums, a sterile cotton swab was dipped into adjusted suspension and rotated several times by pressing firmly on the inside wall of the tube above the fluid level. This removes excess fluid from the swab. Then, the dried surface of Mueller Hinton Agar (MHA) plate was inoculated by streaking using the swab three times over the entire surface by rotating the MHA plates approximately 60° each time to ensure an even distribution of the inoculums. Then, the MHA plates were left open in the hood for three to five minutes to allow for any excess surface moisture to be absorbed (CLSI, 2012).

For the application of the extracts, discs of 6 mm diameter were prepared from sterile filter paper cut into small, circular pieces of equal size by a perforator and then they were impregnated each with 0.01 ml of the prepared test extracts. The extract impregnated discs were placed onto the surface of the inoculated agar plates using sterile forceps. Each disc was pressed down to ensure complete contact with the agar surface. The discs were distributed evenly so that they were no closer than 24 mm from center to center (CLSI, 2012). Discs of commercial amoxicillin (5µg/disc) were used as positive controls while distilled water impregnated discs were used as negative controls. Then the MHA plates were sealed with Para film and incubated at 37°C for 24 hrs for bacterial pathogens. After incubation, the diameters of the zone of inhibition around each disc were measured to the nearest millimeter along two axes (i.e. 90° to each other) using a transparent ruler and the means of the two readings were recorded. For each selected pathogen the experiment was carried out with three replications (Thompson *et al*., 2011; Biswas *et al*., 2013). The experimental design was based on CRD factorial of 3x2x2x4x3 (3 extract concentration levels i.e. 100mg/ml, 150mg/ml and 200mg/ml, (2 solvent system i.e. ethanol and hexane; 2 sources of extracts i.e. tubers and leaf of anchote; 4 bacterial strains and 3 replications) totally, 48 treatment combinations with three replications on zone of inhibition.

3.4.4. Determination of Minimum Inhibitory Concentration

Minimum inhibitory concentration (MIC) is defined as the highest dilution or lowest concentration of the antimicrobial substance (extract) that inhibits growth of organisms. Determination of the MIC is important in diagnostic laboratories because it helps in confirming resistance of microorganisms to an antimicrobial agent and it is useful to monitor the activity of new antimicrobial agents. First nutrient broth (NB) media was prepared by dissolving the required amount of nutrient broth powder with distilled water. Then the mixture was heated on hot plate and autoclaved at 121^{o}C for 15min to get boiled and uniform solution. Then after, Two ml of nutrient broth was added into three test tubes and 0.1 ml of the prepared concentration of each extract that prepared from tuber (ethanol, hexane) and leaf (ethanol, hexane) 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml) were mixed with each test tube containing nutrient broth. Thereafter, standardized inoculum of 0.1 ml of the test pathogen was dispensed into the test tube containing the suspension of nutrient broth and the extract. Then, all test tubes were properly corked and incubated at 37°C for 24 hrs. Then, they were observed for absence or presence of visible growth. The lowest concentration without visible growth of organisms was regarded as the MIC. The experiment was carried out for each organism in triplication (Alabi *et al*., 2012).

3.5. Data Analysis.

Statistical Package for Social Science, Version 20 (SPSS; Chicago, IL, USA), was used to analyze the data. The data recorded from determination of phytochemical contents in leaf and tuber extracts were analyzed by independent sample T-test for comparison while that recorded from antibacterial disc diffusion tests were analyzed using One-way analysis of variance (ANOVA). The significance of output of these statistical tools was considered at $p< 0.05$.

4. RESULTS AND DISCUSSION

4.1. Qualitative Phytochemical Screening

The qualitative test for phytochemicals in Anchote leaf and tuber is presented in Table 1. Except Phlobatannin, the selected class of secondary compounds was detected in ethanolic extracts of both leaf and tuber of anchote. Similar result was also observed in hexane extracted leaf, but alkaloid was lacking in leaf hexane extracts in addition to Phlobatannin. However, tuber extracted in hexane was positive for alkaloid as opposed to leaf extracted in hexane. Little compounds were detected in tuber extracted in hexane. The presence or absence of phytochemicals in one or another solvent's extract provides a very important clue in understanding of their polarity and appropriateness for separation of pure compounds (Gujjeti & Mamidala, 2013). Tiwari *et al*. (2011) and Ugochukwu *et al*. (2013) also reported that successful determination of biologically active compounds from plant material is largely dependent on the type of solvent used in the extraction procedure, and this logic is validated in this study. The same authors report that choice of solvent for extraction depends on quantity of phytochemicals to be extracted, rate of extraction, diversity of compounds to be extracted, diversity of inhibitory compounds extracted, ease of subsequent handling of the extracts, capacity of the solvent in the extracts bioassay process and potential health hazard of the extractant.

In this preliminary phytochemical detection, ethanol extract revealed a positive result in six of the seven tested phytochemicals with the exception of phlobatanin which was absent in both leaf and tuber extract. This shows that ethanol is more effective than hexane to serve as a solvent in this particular case. In general the result of this study shows that although anchote leaves showed high contents of phytochemicals compared to tubers, both organs possess phytochemicals that may be of therapeutic value. Thus, besides the tuber, consumption of the leaves of Anchote can also improve the health status of the consumers.

Reports show that the phytochemicals identified in leaf and tuber part of Anchote have various medicinal properties. For example, alkaloids represent a class of compounds which behaves as diuretic (Haller & Benowitz, 2000). Tannins have amazing stringent properties and known to hasten the healing of wounds, and inflamed mucous membranes. Flavonoids are used as a potent water-soluble antioxidant and free radical scavengers, which prevent oxidative cell damage and

also have strong anticancer activity. In addition it helps in managing diabetes induced by oxidative stress (Yadav *et al*., 2014). Terpenoids are useful in the prevention and therapy of several diseases, including cancer; possess antimicrobial anti-parasitic, antiviral, anti-allergenic and anti-inflammatory properties (Wagner & Elmadfa, 2003). Moreover, terpenoids can be used as protective substances in storing agriculture products as they are known to have insecticidal properties as well (Tiwari & Ata, 2008). Numerous studies have confirmed that saponins possess the unique property of precipitating and coagulating red blood cells Sodipo *et al*., 2000: kumer and Astuti, 211. Steroids are responsible for cholesterol-reducing properties and also help in regulating the immune response (Shah, 2009).

Table 1: Qualitative Determination of Major Phytochemical of *Coccinia abyssinica* extracts

Phytochemical Tested	Tuber Extract		Leaf extract	
	Ethanol	Hexane	Ethanol	Hexane
Tannin	+	+	+	+
Saponin	+	-	+	+
Terpenoids	+	+	+	+
Alkaloids	+	+	+	-
flavonoids	+	-	+	+
Steroids	+	-	+	+
Phlobatannin	-	-	-	-

"+"indicates the presence of the phytochemical while "-"indicates the absence of phytochemical.

4.2. Quantitative phytochemical determination.

Results of quantitative analysis showed that except alkaloid, total phenol, saponin and total terpenoids due to lack of availability material and equipment's that use for quantity analysis. It showed significant variation between organ types and/or solvent used to extract them (Table 2). Although not much pronounced, leaf extract generally seemed to have higher contents of quantified phytochemicals than tuber extract. This may be due to the difference among plant parts in their roles in physiology and survival of the plants under certain circumstances. In supporting of this, Agati *et al*. (2013) reported that plant leaves regulate the antioxidant system

by synthesizing and accumulating more phenolic compounds in the epidermal layers to act as scavengers of reactive oxygen species. On the other hand, Asghari *et al*. (2014) reported that alkaloids are accumulated more in root in some seasons though mainly found in higher amount in leaves during some other seasons. Saponins could be present in different plant parts such as root, tuber, bark, leaf, seed, and fruit (Yongmok & Daniel, 2009). Anchote leaf ethanolic and hexane extracts contained the highest amount 0.389 and 0.355 respective of saponins. However, values obtained in this experiment were lower than what observed in leaves of medicinal plant, *Andrographis neesiana* 1.12, 3.92 (Alagesaboopath *et al.,* 2011). According to Dini *et al.* (2009) the quantity of saponins in food plants can vary considerably due to factors such as variety/cultivar, geographic effects/climate, season, stage of maturity and plant part used. In this experiment, the total phenol content of both organs extracted with both solvent types was found to be lower than some olive cultivars like cassava, carrot and onions which ranged between 8.03, 2.78 and 17.96, 3.94 mg/g (Petridis *et al.*,2012). However, this does not mean that some phenolic compounds that are active against microbes are also lower as total phenolic result shows crude phenolic compounds. Moreover, the total phenol values recoded in both leaf and tuber extracts of anchote in both solvents was lower than leaf extracts of *Adhatoda vasica* 0.395, 0.276 and 0.411, 0.359mg/g respectively, extracted using similar solvents (Maurya and Singh, 2010), but higher than different herb extracts with values 0.181, 0.11 and 0.014, 0.017mg/g ethanol and hexane (Kähkönen *et al*., 1999). The TP content in Anchote tuber were higher than the sweet potato genotypes with distinctive flesh color (Teow *et al*., 2007). In general, the TP content in Anchote ethanol and haxene leaf extract was found to be higher than the TP content vegetables like sweet potatoes and carrots ethanol, hexane extract (0.04 ± 0.0, 0.6 ± 0.1mg/g) TAE (Kähkönen *et al.*, 1999).

In general, comparison of the current study with some that have been previously conducted showed that phytochemical composition and content from the same plant species and plant part varies. The variation may be due to the difference in plant material and extract preparation process such as, extraction methods, percent and volumes of the solvent used, temperature, time, period used for extraction, or concentration or drying of extracts. Accordingly, Agbafer *et al*. (2011) reported that a successful recovery of biologically active compound from plant material is largely dependent on the type of solvent used in the extraction procedure Wendakoon *et al*.

(2012) revealed that bioactive components and contents of plant extracts depend on the concentration of extracts, time period and temperature used in the extraction process.

Table 2: Quantitative Determination of Major secondary compound of *Coccinia abyssinica* extracts

Quantified Phytochemical	Tuber Extracts (mg/g)		Leaf Extracts (mg/g)	
	Ethanol	Hexane	Ethanol	Hexane
Alkaloids	0.363 ± 0.022^{A}	0.344 ± 0.031^{A}	0.363 ± 0.022^{A}	0.346 ± 0.016^{A}
Phenol	0.133 ± 0.012^{AB}	0.113 ± 0.011^{A}	0.265 ± 0.112^{B}	0.222 ± 0.075^{AB}
Saponin	0.387 ± 0.002^{B}	0.350 ± 0.006^{A}	0.389 ± 0.001^{B}	0.355 ± 0.004^{A}
Terpenoids	0.247 ± 0.021^{BC}	0.213 ± 0.012^{A}	0.270 ± 0.017^{C}	0.237 ± 0.015^{AB}

The values are Mean ± Standard error of mean (n=3). Capital letter superscript compares between means in row, and means with similar capital letters represent no significant difference, whereas means with different capital letters are significantly different at $P<0.05$.

4.3. Antibacterial Activity Test

The present study carried out on antimicrobial activities of *Anchote tubers and* leaf extracts in different solvents (ethanol and hexane) on four different human bacterial pathogens namely, gram-positive bacteria (*S. aureus*, *S. pyogenes*) and gram-negative bacteria (*E. coli* and S. *typhi*). The extract ethanol leaf extract and hexane tuber extract of anchote inhibited the growth of both tested gram negative and gram positive bacteria while the hexane leaf extract, ethanol tuber extract and negative control used in the study were not able to inhibit/ no detectable area (0mm ZOI) showed that hexane tuber extract types significantly inhibited the growth of all bacterial pathogen. This difference in growth inhibition between the positive control and the crude extracts indicate the presence antibacterial constituents in the crude extracts of plants which agrees with previous finding on antibacterial activity of the leaves extract of the plant (Murthy *et al.,* 1993). Standard antibiotic (amoxicillin) was used as positive control and caused significantly

the highest zone of inhibition against all tested pathogen compared with those all concentration of both plant part extracts $p< 0.05$ (Table 3).

The antibacterial effects of *Coccinia abyssinica* tubers and leaf crude extracts showed effective bacterial growth inhibition against almost tested bacterial organisms. The Anchote ethanol leaf extract with lowest tested concentration (100mg/ml) showed that low 9.83mm to as high 15.83mm diameter inhibition zones in highest tested concentration 200mg/ml (Table 3). The highest inhibition zone (15.83mm) of Anchote leaf was recorded against *S. aureus*; while the least inhibition zone was seen against *S. aureus* (9.83mm). Similarly, the maximum inhibition zone for hexane tuber extract with the highest tested concentration (200mg/ml) extract seen against *S. aureus* (14.50mm) in while the minimum was against *S. Typhi* (7.83mm) in the lowest tested concentration (100mg/ml).

According to disc diffusion, the growth inhibition of the crude extracts of Anchote ethanol tubers extract and hexane leaf extract with the three tested concentration (100, 150 and 200mg/ml) did not inhibit both gram positive and gram-negative bacteria. On the other hand *Anchote* ethanol leaf and hexane tuber crude extracts with the lowest tested concentration (100mg/ml) was inhibitors against *S. aureus* (9.83mm) and *S. typhi* (7.83mm). The inhibition of crude extracts against *S. typhi, E. coli, S. pyogenes* and *S. aureus* were significantly smaller than the inhibition of positive controls with all the tested different concentration. When the Concentrations of extract used compare with positive controls, positive controls were much more potent than the extracts used in the present study.

The crude extracts of Anchote leaf with the highest tested concentration (200mg/ml) on *S. aureus* inhibited significantly greater area than against *E. coli, S. typhi* and S. pyogenes. Among tested bacterial species *S. typhi* and *E. coli* showed lesser susceptibility to crude extracts of tubers of (ethanol, hexane), respectively. These could be because of inherent ability of this bacterial species to produce resistance mechanisms like efflux pump, reduced permeability or biofilm formation which could hinder the antibacterial activity of the bioactive compounds (Habtamu and Waktola, 2014). Inhibition area of the crude extracts increased with concentration, which implies that the antibacterial activity of the crude extracts were dose dependent; this proportional increase in inhibitory activity as the concentration of extract increase were also reported in other study on other plants (Yadav *et al*., 2011).

Among the test bacterial strains, gram positive bacteria were more susceptible to the crude extract. The mean zones of inhibitions of the crude extracts were higher for gram positives than gram negatives at their comparable concentration. These differences in susceptibility between gram positive and gram negative bacteria could be because of their difference in their cell wall composition (Nikaido and Vaara, 1985). The gram negative bacteria lipopopoly saccharide rich outer cell membrane may partially hinder the passage of active phytochemicals to the cell unlike gram positive which have no outer cell membrane. Moreover, the crude extracts showed antibacterial activities against both gram positive and gram negative bacteria which could indicate that the presence of broad spectrum bioactive metabolites in the study plant (Srinivasan *et al*., 2001).

Different solvents' extracts (ethanol leaf and hexane tuber) extracts of anchote showed different antimicrobial efficiency. Ethanol leaf extract was the most active and showed considerable antibacterial activity against tested gram positive and gram negative bacteria producing a maximum inhibition zone of 15.83mm against *S. aureus* with the highest tested concentration (200mg/ml). Similarly, the minimum inhibition zone of 9.83mm against *S. aureus* was observed with the lowest tested concentration (100mg/ml). The relatively high anti-staphylococcal activity might, however, be of interest since several *Staphylococcus* strains are reported to express a multi-drug resistance and natural plant extracts were shown to have good anti-staphylococcal activity (Lechner *et al*., 2004). Hexane tubers extract was the less active and showed considerable antibacterial activity both tested gram positive and gram negative bacteria producing a maximum inhibition zone of 14.50mm against *S. aureus* with the highest tested concentration (200mg/ml) and the minimum inhibition zone of 7.83mm against *S. typhi* with the lowest tested concentration (100mg/ml).

This result showed that different solvents have a different capacity of extracting active principles to inhibit microbial growth. Measured inhibition zones of 15.83, 14.50mm (ethanol leaf and hexane tuber) extracts, respectively were related with zone diameter of standard drugs Amoxicillin (17.00mm, 16.17mm) for bacterial strains. This may be due to the extract has different bioactive compounds that particularly enhance antimicrobial activities of plant extracts (Kedarnath *et al*., 2012). These support the strong scientific basis for the use of these plants in the traditional treatment of human infectious diseases. The observed different result from current

study on antibacterial activities of *Coccinia abyssinica* may be due to using different solvent for extraction, the strain of pathogen that were tested, bacterial morphology and environmental condition and types of obtained chemical composition in Anchote (Kumar *et al.*, 2015).

Table 3: Zone of inhibition of selected bacterial pathogens treated with Anchote extract

Antimicrobial agents	*Concentration in mg/ml*	Zone of Inhibition (ZI) in mm			
		E. coli O157	*S.* Typhi	*S. aureus*	*S. pyogene*
Ethanol Leaf extracts	100	12.17 ± 0.29^{Bb}	10.33 ± 0.29^{Ac}	9.83 ± 0.76^{Aa}	10.17 ± 0.29^{Ab}
	150	14.60 ± 0.17^{Bc}	11.83 ± 0.76^{Ad}	14.43 ± 0.40^{Bc}	11.47 ± 0.42^{Acd}
	200	14.83 ± 0.76^{Bc}	13.17 ± 0.29^{Ae}	15.83 ± 0.76^{Bd}	12.17 ± 0.29^{Ad}
Hexane Tuber extracts	100	8.70 ± 0.26^{Ba}	7.83 ± 0.29^{Aa}	9.50 ± 0.50^{Ca}	8.83 ± 0.29^{Ba}
	150	11.40 ± 1.44^{BCb}	9.40 ± 0.66^{Ab}	13.00 ± 0.50^{Cb}	10.90 ± 0.79^{ABbc}
	200	13.67 ± 0.76^{Bc}	10.50 ± 0.50^{Ac}	14.50 ± 0.50^{Bc}	14.23 ± 0.40^{Be}
Amoxicillin	0.5µg	17.00 ± 1.00^{BCd}	15.67 ± 0.58^{Af}	17.50 ± 0.50^{Ce}	16.17 ± 0.29^{ABf}
Distilled water		-	-	-	-

Capital letters compare means between row and small letters compares means between columns. Means with different capital and small letters are significantly different at ($p<0.05$).

4.4. Minimum Inhibitory Concentration

As shown in Table 4, the minimum inhibitory concentration of the crude extracts from both ethanol leaf and hexane tuber of Anchote ranged from 41.67 – 100 mg/ml and those of against the test pathogens. The smallest MIC of the crude extract was for both S. *aureus and S. payogene* which was 41.67mg/ml of ethanol leaf and hexane tuber extract. This result agrees with the finding of Patrone and Stain (2007), which indicated that gram positive bacteria were more susceptible than gram negative bacteria.

The ethanol leaf extract of Anchote had inhibited the growth of test pathogens at concentrations lower than that hexane extract of tuber Anchote. The MIC values of ethanol leaf of Anchote were 50mg/ml and 66.67mg/ml for *E. coli and S. typhi,* respectively; and 41.67mg/ml for *S. aureus and S. pyogene.* In contrast the MIC values of hexane tuber extract higher than those of

ethanol leaf extract. The data also show that gram positive bacterial pathogens were inhibited with concentrations higher than those gram negative bacterial species. The gram positive strains such as *S. aureus*, *S. pyogene* and gram negative strains such as *S. typhi and E. coli* causing serious infection in human and in other animals including superficial skin lesion, localized abscesses, and food poisoning were in first positions (Topley and Wilsons, 1998).

In this study, the *Coccinia abyssinica* exerted antibacterial activity against both Gram positive and Gram negative bacteria associated with different type of infections including pneumonia (k. pneumonia), urinary tract infections (*S. aureus*) and wound infections. The demonstration of activity against both Gram positive and Gram negative bacteria is an indication of broad spectrum of activity and thus can be used as source antibiotic substances for drug development that can be used in the control of these bacterial infections. The *Coccinia abyssinica* plant was used for testing their antibacterial activity and showed high activity against those organisms such as *S. aureus, E. coli, S. typhi* and *S. pyogene.* These indicate that the herbal preparations could be used for preventing and treat the diseases caused by those selected organisms.

Table 4: Minimum Inhibitory Concentration at the lowest concentration (100mg/ml) of Anchote extracts against selected bacterial pathogens

Selected Pathogens	Minimum Inhibitory Concentration in mg/ml	
	Ethanol Leaf extracts	Hexane Tuber extracts
E. coli O157	50±0.00 (50, 50 and 50)	66.67±28.87 (50,50 &100)
S. Typhi	66.67±28.87 (100, 50 & 50)	100±0.00 (100,100 &100)
S. aureus	41.67±14.43 (50,25 & 50)	41.67±14.43 (25,50 & 50)
S. pyogene	41.67±14.43 (50, 25 & 50)	41.67±14.43 (25,50 & 50)

5. SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

5.1. Summary and Conclusions

Anchote (Coccinia abyssinica), tubers and leaf are valuable sources of nutrients and bioactive compounds for treatment of common and infectious diseases. Therefore, the aim of this study was to quantify bioactive compounds and phytochemical screening by following standard procedure and quantity of screened crude alkaloids, terpenoids and saponin. Besides, the screening out total phenolic content in the tubers and leaf extracts of both plants were determined using a technique of spectrophotometric method. The results of present study evidenced that studied (ethanol, hexane) extract from the leaf and tuber of *Anchotes* have the ability to inhibit the growth of the selected bacterial pathogens with its different type of secondary metabolites. The results of qualitative phytochemical screening of leaf (ethanol, hexane) and tuber (ethanol, hexane) of anchote extract revealed the presence of alkaloid, saponin, flavonoid, steroid, tannin and terpenoids. Whereas, phlobatannins was absent in both plants tuber and leaf of Anchote.

The presence of these phytochemical provided basis for the traditional uses of these plants against some diseases. Regarding the efficiency of extracting solvents, qualitative phytochemical screening of leaf and tuber extracts of anchote was done. The result the presence tannin, terprnoid and alkaloids both in ethanol tuber and hexane tubers extract. Tannin, saponin, terpenoid, flavonoid and steroid were present in ethanol leaf and hexane leaf extract. Saponin, flavonoid and steroid were absent in hexane tuber extracts and alkaloids were absent in hexane leaf extract. Quantitative analysis showed that generally leaf (ethanol and hexane) extracts had the highest concentration of phytochemicals. The concentration of alkaloid (0.363, 0.346 mg/g), terpenoids (0.270, 0.237mg/g), phenolic (0.265, 0.222mg/g) and saponin (0.389, 0.355 mg/g) were found in larger in leaf extract than tuber extract.

Additionally, the present study was aimed to evaluate antimicrobial activities of *Anchote tubers* and leaf extract separately in different solvent (ethanol and hexane) against some human pathogens namely *E. coli* O157 H:7 (ATCC 25922), *Salmonella typhi* (ATCC 13311), *Staphylococcus aureus* (ATCC 25923) and *Streptococcus pyogenes* (ATCC 196151). Disk diffusion method was used to test antimicrobial activity for the ZOI of anchote extracts and MIC concentrations were determined by using broth dilution method. The result of antibacterial activity test showed that tuber and leaf (ethanol and hexane) extracts shows different degree of

inhibition against different isolates except hexane leaf and ethanol tuber extracts with no inhibition against human pathogenic strain. Both ethanol leaf and hexane tuber extracts have the most antibacterial activity against *S. aureus*. Antibacterial activities of both extracts were significantly less than that of amoxicillin which was used as positive control. The ethanol leaf and hexane tuber extract of this medicinal plants were significantly active against Staphylococcus species (Selvamohan *et al*., 2012).

Generally, the antimicrobial activities and phytochemical screening of anchote extracts showed significant antimicrobial activity and phytochemical constituents showed most of the antibacterial activities (Biswas *et al*., 2013). The present study shows that the tuber (ethanol, hexane) and leaf (ethanol, hexane) of *anchotes* have all the phytochemical constituent studied with negligible variation. Thus, the significant activity against both gram positive and gram negative bacterial pathogens may be due to their phytochemical or secondary metabolites.

5.2. CONCLUSIONS

Generally, it is concluded that this study would lead to the establishment of some valuable compound that has to be used to formulate new, different and more potent antimicrobial drugs of natural origin. Phytochemical screening was carried out on the anchote tuber and leaf (ethanol, hexane) extract which revealed the presence of alkaloids, steroids, tannins, saponins, flavonoid and terpenoids. Phlobatanin were absent.

The plant based products have been effectively proven for their utilization as source for antimicrobial compounds (Lechner *et al*., 2004). The crude extracts of *Coccinia abyssinica* exhibited moderate to significant antibacterial activity against tested bacteria with inhibition zone and comparable to positive control. Ethanol leaf extract was the most active and showed considerable antibacterial activity against tested gram positive and gram negative bacteria producing a maximum inhibition zone of 15.83 mm against *S. aureus* at 200mg/ml concentration and a minimum inhibition zone of 12.17 mm against *S. pyogene.* Hexane tuber extract was the most active and showed considerable antibacterial activity against tested gram positive and gram negative bacteria producing a maximum inhibition zone of 14.50 mm against *S. aureus* at 200mg/ml concentration and a minimum inhibition zone of 10.50 mm against *S. typhi.* No activity was shown by hexane leaf and ethanol tuber extract against the tested bacteria with inhibition zone. The highest minimum inhibitory concentration MIC of ethanol leaf and hexane tuber extracts observed against *S. typhi* and *E. coli* and the lowest MIC against *S. aureus* and *S. pyogenes.*

The antimicrobial activities of plant crude extracts depend on the dose, types of solvents and type of test organisms. The strong antimicrobial activities of ethanol leaf extracts and hexane tuber extract of anchote was correlate well with the use of this plants in traditional medicine for treatments of gonorrhea, tuberculosis and cancer. Therefore, the ethanol and hexane extracts from leaf of anchote and hexane tuber extract could be a good source for useful drugs.

5.3. Recommendations.

Based on the scope and results of the present study, the following recommendations were forwarded:

- ✓ The finding of this study has indicated the leaf extract of anchote was potential for treatment of *Staphylococcus aureus*. Therefore, community should be encouraged to include anchote leaf in to their food stuff.
- ✓ Ethanol is recommended for the extraction of antimicrobial phytochemical of anchote tubers and leaf.
- ✓ Leaf extract of ethanol is a better source of further extraction and purification of saponin for large scale therapeutic use.
- ✓ Further studies are needed to identify the specific biologically active compounds and to evaluate the efficiency of the compound against pathogenic microorganisms associated with various human diseases.
- ✓ Effective isolation and detailed characterization of the different phytochemicals and in vivo antibacterial activity against selected pathogens using model animals should be conducted.

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7. APENDIX

Fig.1.During sample collection process

Fig.2.During grinding and result of qualitative phytochemical analysis

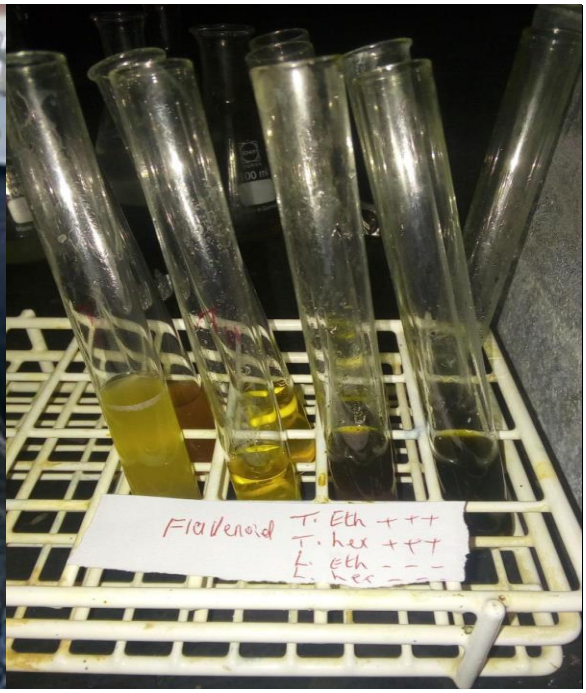

Fig.3. During preparation of different concentration and antimicrobial activities work

Fig.4. Result of antimicrobial activity

Figure 5: standard curve of Tannic acid